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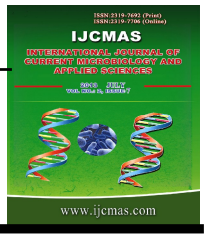

Original Research Article

Biodiversity and Biodegradation potentials of Fungi isolated from Marine systems of East Coast of Tamil Nadu, India

R. Saravanan and T. Sivakumar*

Department of Microbiology, Kanchi shri Krishna College of Arts & Science, Kilambi -631 551, Kancheepuram, Tamil Nadu, India
**Corresponding author: shiva_fungi@rediffmail.com*

A B S T R A C T

The present study was confined to the marine ecosystem in and around Mamallapuram coast, Tamil Nadu comprising of Mammallapuram and Water, sediment, and natural substrates of marine ecosystem were collected to isolate the fungi. All the collected samples were plated, incubated and the fungal colonies were identified. The water and sediment sample were collected separately and analysed for temperature, pH, biological oxygen demand, salinity and total dissolved solids on water. A total of 41 fungal species were isolated and enumerated by plating, techniques. In this study, 24 species of fungi were recovered from sediment samples whereas water samples yielded 30 species and natural substrates with 24 species. Among the Hyphomycetes, *Aspergillus* was the common genus represented by 14 species followed by *Penicillium* and *Cladosporium*. Totally, 10 species of fungi were used for hydrocarbon studies. The growth pattern of fungi in diesel is *T.viride* had a maximum growth 24mm with *A. sulphueus* had the least growth growth on the 5th day at 9 mm with *A.niger*. The growth pattern of fungi in petrol is *P.citrinum* had maximum growth on the 5th day at 90mm and minimum with 9 mm by *R.oryzae.* The growth pattern of fungi in Crude oil shown *A.flavus* had a maximum growth at on the 5th day mm and minimum growth rate was observed in *A.oryzae* with 9 mm. The growth pattern of fungi in kerosene is *P.janthinellum* had a maximum growth at 27 mm and the lowest growth rate in *A. oryzae* with 5 mm.

K e y w o r d s

Marine fungi; Isolation of fungi; Physico-chemical parameters; Species Diversity; Hydrocarbon degradation.

Introduction

Biological diversity refers the variability among living organisms from all sources including terrestrial, marine and other aquatic ecosystem and ecological complexes of which they are part. Biodiversity encompasses all life forms, ecosystems and ecological processes and acknowledges the hierarchy at genetic, ingredients of biodiversity are phenotypic flexibility genetic variation within populations and ecotypic variations (Ananthakrishnan, 1997). Microbial biodiversity can be viewed from a variety of perspectives, including physiological diversity, interaspecific genetic diversity and phylogenetic diversity of species and

higher taxa (Delong, 1997). Microbial diversity represents the largest untapped reservoir of biodiversity for potential discovery of new biotechnological products, including new pharmaceuticals, new enzymes, new special chemicals or new organisms that carryout novel process (Jensen and Fenical, 1994). Quantitative data on the occurrence of tropical marine fungi have been published by Koch (1986); Kohlmeyer (1984); Zainal and Jones (1984, 1986). However all of these reports were on driftwood in the sea, along with driftwood on the mangrove floor or panels belonging to various timbers submerged near jetties.

The marine fungi of Hong Kong and Thailand have been studied intensively over the past 15 years, and include not only random collections of drift material, but also the exposure and recovery of bait samples (exposure of bait in Hong Kong (Vrijmoed *et al.,* 1986; Sadaba *et al.,* 1995; Abdel- Wahab, 2000; Thailand: Pilantanapak *et al.,* 2005), collection of drift and attached mangrove samples in Hong Kong (Abdel-Wahab and El-Sharouny, 2002; Jones and Vrijmoed, 2003), Thailand (Hyde *et al.,* 1993; Sakayaroj *et al.,* 2004). Schmidt and Shearer (2004) analysed the geographical distribution data published on lignicolous mangrove fungi, and found that different oceans supported varying numbers. The number of fungi at each site varied: Atlantic Ocean: 12-46 per site (14 sites: mean 25.6); Indian Ocean: 12-64 (14: 42.9) and the Pacific Ocean: 17-87 (16: 44). The Pacific Ocean has the highest recorded number of fungi, again the result of repeated collections over many years: Hyde (1988c) in Brunei; Jones and Kuthubutheen (1989), Alias *et al.* (1995), Tan *et al*., (1989) and Leong *et al.,* (1991) in Singapore, and the greater diversity of mangrove tree species in this region. The paucity of marine fungi from the Atlantic has been attributed to low mangrove tree diversity, for example three in Florida mangroves and four in the Bahamas (Jones and Abdel-Wahab, 2005). However, more intensive collections yielded 81 species for Florida mangroves from 250 collected samples and 112 for the Bahamas from 600 collected samples, where only 31 had previously been recorded (Jones and Abdel-Wahab, 2005).

Petroleum like all fossil fuels primarily consists of a complex mixture of molecules called hydrocarbons. In large concentrations, the hydrocarbon molecules that make up crude oil and petroleum products are highly toxic to many organisms, including humans (Alexander, 1994). The dominance of petroleum products in the world economy creates the conditions for distributing large amounts of these toxins into populated areas and ecosystems around the globe (Ojumu, 2004). The most rational way of decontamination of the environment loaded with petroleum derivatives is an application of methods based mainly on metabolic activity of micro organisms (Leahy and Colwell, 1990). Microbial degradation is the major mechanism for the elimination of spilled oil from the environment (Ibe and Ibe, 1984; Atlas, 1995). The ability to actively decompose specified fractions of petroleum oil is expressed by many micro organisms (Bartha and Atlas, 1977). Yuan et al (Yuan, Wei and Chang, 2000) suggested introduction of mixed cultures of bacteria and fungi, especially that not all components of petroleum – derived hydrocarbon mixture are decomposed simultaneously. However, single cultures of fungi have been found to be better than mixed cultures (Okerentugba and

Ezeronye, 2003) and more recently, fungi have been found to be better degraders of petroleum than traditional bioremediation techniques including bacteria (Batelle, 2000). Based on the necessary basic information obtained on marine fungi ecosystem, the present study has been undertaken in the proposed study area in Mammallapuam, a coastal deltaic habitat along the East coast of Palk Strait, in Bay of Bengal in Tamil Nadu, India.

Materials and methods

Study area

Totally five (5) sampling stations were selected in eadt coastal areas of Mammallapuram. Water, sediment and natural substrates of plants were collected From the sampling stations.

Isolation of fungi from water and sediment samples by plating technique

After sampling, within 24 hrs the water and sediment samples from each station were subjected to appropriate dilutions (10^{-2} to10^{-5}) and 0.1 ml of sample was aseptically transferred into the plates containing Potato dextrose agar, Rose Bengal agar with addition of mixture antibiotics, Tetracycline and Penicillin (Spread plate method) The plates were incubated at room temperature (28°C) for 4-5days. Control plates were also maintained. Sterilization of glasswares and preparations of media were carried out as per the method described by Booth (1971).

Isolation of fungi

The incubated plates were observed for the development of fungi from 3rd day onwards. The number of colonies in each plate was counted and compared with control. In addition to this, cultural characters of the colonies (color and structure) were also observed and fungi were enumerated. The natural baits kept in the plates were observed directly under the Stereoscopic Binocular dissection Microscope from 5th day onwards.

Presentation of data

The semi permanent slides of the isolated fungi were prepared using Lactophenol Cotton Blue Staining method (Dring, 1976**)** and sealed with DPX mountant. The fungal species were photographed using photo micrographic instrument (Nikon AFX II Microscope fitted with Nikon FX-35 camera, Tokyo, Japan).

Identification of fungi

The identification of fungal taxa was based on Hyphomycetes **(**Subramanian, 1971), Dematiaceous Hyphomycetes and More Dematiaceous Hyphomycetes (Ellis, 1971, 1976), Marine Mycology (Kohlmeyer and Kohlmeyer, 1979), Micro fungi on land plants (Ellis and Ellis, 1985) Micro fungi on Miscellaneous substrate (Ellis and Ellis, 1988), Illustrated key to the filamentous higher marine fungi (Kohlmeyer and Volkman - Kohlmeyer, 1991) and Manual of soil fungi (Gilman, 1957, 1998).

Maintenance of fungal cultures

Cultures are maintained by separated sub culturing on appropriate medium. Fungi cultures were inoculated into the PDA plates. The plates were maintained at room temperature for week.

Physico – chemical analyses of water and sediment samples

The water and sediment sample were collected separately and analysed for

temperature, pH, dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), salinity, total dissolved solids (TDS) on water (Venugopalan and Paulpandian,1989; Aneja, 2001; APHA, 1998) .

Biodegradation of hydrocarbon study

Toally 10 species of fungi were selected based on frequency of occurrence for hydrocarbon degradation.

Determination of the Fungi Hydrocarbon Growth Capability

Qualitative determinations of the fungi growing on hydrocarbon incorporated PDA media were determined by culturing of fungi. Four concentrations of oil-contaminated PDA media (5%, 10%, 15% and 20% (v/v) of kerosene) and one control PDA media (non-oil-contaminated) were prepared in twenty petri dishes and isolated strains were inoculated in each. All the petri dishes were then incubated at room temperature (28°C - 31°C) for 10 days. After 10 days, the control and radially growing colony were examined for the growth diameter. Similarly mixers of all the four species were used for the assay.

Results and Discussion

The results of study in marine ecosystem comprising of Mammallapuram are presented and discussed under three sections, *viz*., Enumeration of taxa, Ecology of fungi, andBiodegradation potentials of fungi.

Enumeration of taxa

The fungi belonging to different genera which were isolated by plating and baiting techniques were enumerated with morphological and ecological descriptions. The system of classification was based on "The Fifth Kingdom – Mycota (ed.) Kendrick (1992) for the arrangement of genera under their respective orders and families. The genera and species within each family are arranged in alphabetical order.

Ecology of fungi

This section deals with the ecology of fungi include Physico-chemical status of water and sediment samples with respect to fungal distribution, Species diversity fungi in the mangrove ecosystem, fungal distribution in relation to mangrove vegetation and Biodegradation of hydrocarbons. The ecology of fungi in a marine system depends on the various physical, chemical and biological factors of the water and sediment samples. In the present investigation, a study has been made on the distribution of fungi in relation to sampling stations, marine vegetation, frequency, and physico-chemical nature of the marine system.

Physico-chemical status of water and sediment samples with respect to fungal distribution

Hence, 5 parameters *viz*. temperature, pH, biological oxygen demand (BOD), salinity, and total dissolved solids (TDS) of water samples were observed and recorded (Table.1). The physico-chemical parameters of water samples in all stations, salinity, BOD, TDS, were 44.0 mg, 16.2mg/l, 42.24 mg respectively in S1. Salinity and TDS were 52%, 42.08 mg respectively in S2. These parameters were influenced the occurrence of fungi which belonged to Zygomycotina, Ascomycotina and Deuteromycotina.

Table.1 Details of physico-chemical parameters of water in five stations.

Parameters	S1	S2	S3	S4	S5
Temperature (° C)	28	29	30	28	28
pH	7.8	8.0	8.1	8.2	7.5
Biological oxygen demand (mg/l)	16.2	18.1	13.2	14.1	15.3
Salinity (%)	44	52	40	43	42
Total dissolved solids (mg/l)	42.24	42.08	41.94	44.09	44.31

The physico-chemical parameters of water in S3 stations as follows: BOD (13.2mg), salinity (40%) total dissolverd solids (41.94 mg). Table 1 shows, the parameters of water S4 stations salinity, total dissolved solids, were 43%, and 44.09 mg respectively. The physico-chemical parameters of water in S5 stations as follows: BOD (15.3mg), salinity (42%) total dissolverd solids (44.31 mg). The parameters influenced the occurrence of 41 fungi, belonging to Zygomycotina, and Deuteromycotina.

Species diversity of fungi in the mangrove ecosystem

During the four month study period, a total of 41 fungal species were enumerated from five sampling stations S1, S2, S3, S4, and S5 by plating and baiting techniques. Among these, 12 species were represented in S1, 12 in S2, 15 in S3, 12 in S4 and 16 in S5. (Table 2). Maximum fungal diversity was observed in S5 with represented by 16 species and minimum of 12 species was isolated in S1, S2 and S4. In this study, 24 species of fungi were recovered from sediment samples whereas water samples yielded 30 species and 24 species were isolated from natural substrates (Table 3).

When the fungal species diversity was analyzed in relation to different classes, it has been observed that the maximum number of species recorded belonged to Hyphomycetes. This was followed by Zygomycetes and Ascomycetes . Among the Hyphomycetes, *Aspergillus* was the common genus represented by 14 species followed by *Cladosporium* with 5 species, 6 species with *Penicillium* and *Fusarium* with 3 species. In addition to this *Mucor*, *Rhizopus*, were the common genera found in this marine system.

Occurrence of fungi in the water

In this study, totally 24 species of fungi were isolated and enumerated from the water samples by dilution-plating technique. Of all these, *Aspergillus* were found to be dominate genus with 13 species, followed by *Penicillium* (4 species), *Curulanata* (3 species). The above result was discussed with previous reports of Chandralata (1999) and Raghukumar and

Table.2 Fungi isolated from all the five sampling stations and marine substrate

Name of the fungi	Sampling stations					Marine Substrate		
	S1	S2	S3	S4	S5	Water	Sediment	Natural Substrates
Muco muceda	-	-	-	+	-	+	-	-
Mucor sp.	-	-	+	-	-	+	-	+
Rhizopus oryzae	-	+	+	-	-	-	+	+
R. nigricans	+	-	-	-	-	-	+	+
R. stolonifer	-	-	-	+	-	-	+	-
Saccharomyces sp. 1	+	+	+	-	+	+	+	-
Neurospora crassa	+	+	-	+	-	+	+	-
A.alliaceus	-	-	-	-	-	+	-	+
A.awamori	-	-	-	-	+	-	+	-
A. carbonarius	-	-	-	-	+	-	-	+
A. erthrocephalus	-	+	-	-	-	-	+	-
A. flavus	-	+	+	+	+	+	+	+
A. fumigatus	+	+	-	-	+	+	+	+
A.funiculosis	-	-	-	-	-	-	-	+
A. luchuensis	-	-	+	+	+	+	+	+
A. nidulans	+	-	+	-	-	-	+	-
A. niger	+	-	+	+	+	+	+	+
A. ochraceus	-	-	+	+	+	+	+	+
A. oryzae	+	+	+	+	-	+	+	+
A. sulphureus	+	-	-	-	+	+	+	-
A. terricola	-	-	-	-	+	+	-	+
A. terreus	+	+	+	-	+	+	+	-
Pencillium citrinum	-	-	-	-	+	-	+	-
P. janthinellum	+	-	-	-	+	+	+	-
P. purpurrescens	-	-	-	-	-	-	+	+
Penicillium sp.1	-	+	-	-	-	-	+	-
Penicillium sp.2	-	-	-	-	-	-	+	+
Trichoderma viride	-	-	+	-	-	-	+	+
Alternaria sp.	-	-	-	-	-	-	-	+
Cladosporium apicale	-	+	-	-	-	+	-	+
C. britannicum	+	-	-	-	-	-	+	-
C. tenuissimum	-	-	-	+	-	-	+	+
Cladosporium sp.1.	+	-	-	-	-	+	+	-
Cladosporium sp.2	-	+	-	-	+	+	+	-
Cladosporium sp.3	-	-	+	+	-	+	+	-
C. lunata	-	-	-	+	-	+	-	-
C.subluta	-	-	+	-	-	-	+	-
Curvularia sp.	-	-	-	-	+	+	-	+
F. oxysporum	-	-	-	-	-	-	+	+
F. semitectum	-	-	-	+	-	+	-	-
Fusarium sp.	-	-	-	-	-	+	-	+

(+) – Present, (–) - Absent

Raghukumar (1998) also reported adaptation and activity of terrestrial fungi under marine/ mangrove ecosystem as facultatives or indwellers or residents. Terrestrial fungi are common in mangrove water and mud (Chowdhery *et al*., 1982; Garg, 1983). Seawater, seafoam and beach soil of Arabian Gulf Coast, Saudi Arabia yielded terrestrial fungi, typical marine and freshwater fungi (Bokhary *et al*., 1992).

Occurrence of fungi in the sediment

By employing the plating technique, 30 fungi were isolated from the mangrove sediment samples. As like in the water samples, in sediments samples also the genus *Aspergillus* was also found to be dominant followed by *Cladosporium.* With the above-presented results while, assessing the species diversity of fungi in the estuarine waters and sediments, the fungal genera, *Aspergillus, Penicillium, Curvularia, Alternaria, Cladosporium* and *Drechslera* were found to be dominant members of this system. This well agreed with the findings of Garg (1982), Rai and Chowdhery (1978) and according to their findings Aspergilli dominated over Mucorales and Penicillia in the mud of mangrove swamps of Sunderband.

Sparrow (1934, 1936) reported that the presence of *Aspergillus* and *Penicillium* species in the marine sediments. Satio (1952) investigated the mycoflora of a salt marsh and observed that the species of *Penicillium* and *Trichoderma vignorum* were the common forms encounted in the surface mud. This well correlates with the findings made by Garg (1983) in which, he came comecross highest number of fungi from surface layer of the Sunderban mangrove mud.

Isolation of *Aspergillus* species in greater number and frequency is due to the high nutrient level in the mangrove eco-system. These species prefer a medium with high osmotic concentration and therefore, compete more easily with other forms in the mangrove eco-system.

Distribution of fungi in relation to marine vegetation and their substrates

The fungi in the marine system were studied by plating and baiting techniques at certain specific sampling stations where, the plant vegetation was dense and varied. Totally, 24 species of fungi belong to different groups were enumerated from the natural substrates wood,algae, attempted with direct plating techniques (Table.3). *Aspergillus* was found to be more predominant fungi, *A. flavus, A. fumigatus, A. luchuensis, A. terreus, A. nidulans*, followed by *Penicillium* sp**.**

Mangrove vegetation plays an important role in the distribution of fungi in the aquatic system, since they contribute to the leaf litter which harbor mycoflora. Fungi which occur on driftwood, intertidal wood, manalia rope and other lignocellolic substrates in marine and estuarine environments have been reported by Johnson and Sparrow, (1961), Kohlmeyer and Kohlmeyer (1979).

Biodegradation of hydrocarbon studies

Totally, 10 species of fungi were isolated from marine samples and used for hydrocarbon studies. The growth pattern of fungi in diesel is represented on Table.4. *T.viride* had a maximum growth 24mm with *A. sulphueus* had the least growth growth on the 5th day at 9mm with *A.niger*. The growth pattern of fungi in

Table.4 Biodegradation of hydrocarbon (Growth rate of fungi)
(The values are represented in mm)

S.No	Name of the fungi	Diesel	Petrol	Crude oil	Kerosine
1	*A.niger*	9	21	40	15
2	*A.flavus*	10	41	86	10
3	*F.semitectum*	20	31	38	21
4	*P.janthinellum*	16	30	36	27
5	*A.oryzae*	12	22	9	5
6	*A.ochraceous*	19	61	17	40
7	*P.citrinum*	16	90	47	91
8	*A.sulphreus*	24	28	83	15
9	*R.oryzae*	19	9	38	15
10	*A.terricola*	17	29	33	15

petrol is shown in table 9. *P.citrinum* had maximum growth on the 5th day at 90mm and minimum with 9 mm by *R.oryzae*. The growth pattern of fungi in Crude oil is represented in table 10. *A. flavus* had a maximum growth at on the 5th day mm and minimum growth rate was observed in *A.oryzae* with 9mm. The growth pattern of fungi in kerosene is represented on Table.11. *P. janthinellum* had a maximum growth at 27 mm and the lowest growth rate in *A.oryzae* with 5 mm (Table 4).

The results of this work indicate that many of the fungal species isolated from the marine system were capable of degrading petroleum hydrocarbons. Bartha and Atlas (1973) listed 22 genera of bacteria, 1 algal genus and 14 genera of fungi which had been demonstrated to contain members which utilize petroleum hydrocarbons; all of these micro organisms had been isolated from an aquatic environment. Also, Okerentugba and Ezeronye (2003) demonstrated that *Penicillium* spp., *Aspergillus* spp. and *Rhizopus* spp. were capable of degrading hydrocarbons especially when single cultures were used. These fungi had been isolated also from aquatic environments in the Niger Delta area of Nigeria. Batelle (2000) showed that fungi were better degraders than traditional bioremediation techniques including bacteria. The fungi used were wood-degrading fungi. The isolation of fungal petroleum hydrocarbon utilizers from oil seeds was first documented by Adekunle and Oluyode (2002).

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